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EFFECT OF PSEUDOMONAS AERUGINOSA EXOTOXIN-A ON THE SYNTHESIS AND SECRETION OF PROTEINS IN ISOLATED RAT PANCREATIC ACINI

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Exposure of isolated rat dispersed pancreatic acini to increasing concentrations (10 to 1000 ng/ml) of purified exotoxin-A from Pseudomonas aeruginosa resulted in a progressive inhibition of ^{3}H-leucine incorporation into "cellular" (those remaining in the cells) and "secretory" (those released into the medium) proteins. With each concentration of exotoxin-A, magnitude of reduction was found to be greater for the "secretory" proteins than that observed for the "cellular" proteins. Thus, in the presence of 250 ng/ml of exotoxin-A, a dose that produced maximal inhibition in protein synthesis, ^{3}H-leucine incorporation into "cellular" and "secretory" proteins was found to be decreased by about 19 and 50%, respectively, when compared with the corresponding basal controls. Release of trypsinogen, chymotrypsinogen and amylase from the isolated pancreatic acini was also inhibited by high doses of exotoxin-A. However, whereas the exotoxin concentration of 1000 ng/ml, caused a near complete inhibition of chymotrypsinogen release, trypsinogen and amylase secretion were decreased by 40 and 50%, respectively. It is concluded that in isolated pancreatic acini, exotoxin-A inhibits the synthesis and secretion of proteins.

Pseudomonas aeruginosa exotoxin-A constitutes on a weight basis the most potent exoproduct of this opportunistic pathogen and is produced by about 90% of clinical isolates. Its mean lethal dose (LD_{50}) is 60-80 ng per mouse (1,2). Pseudomonas exotoxin-A has been shown to be cytotoxic to a number of cell lines. Pavlovskis and Gordon (3) have shown inhibition of uptake of ^{14}C-uridine and ^{14}C-amino acids by exotoxin-A on Vero cells (African green monkey-kidney cells). Iglewski and Kabat (4) have reported that pseudomonas exotoxin-A inhibits protein synthesis in a rabbit reticulocyte lysate, and Leppla (5) has shown that it blocks amino acid incorporation in a wheat germ cell-free system. In addition, it should be mentioned that in mice, a single

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intravenous injection of pseudomonas exotoxin-A results in a 50% inhibition in hepatic protein synthesis within 4 h and virtually complete inhibition occurs before the time of death (6). However, no cytotoxicity studies with pseudomonas exotoxin have been reported to date with two well known in vitro systems mainly isolated hepatocytes or dispersed pancreatic acini. The exocrine pancreatic cell possesses a very high rate of protein synthesis and secretes on demand about 20 proteins mainly enzymes and zymogens. The dispersed pancreatic acini have been employed in this study, in order to determine the cytotoxicity of Pseudomonas aeruginosa exotoxin-A as reflected by its effect on the rate of synthesis and secretion of certain enzyme-proteins.

MATERIALS AND METHODS

Dispersed pancreatic acini were isolated from 18 h fasted adult male Sprague-Dawley rats (200-250 g) with a slight modification (7,8) of the procedure described by Williams et al (9). Routinely, acini isolated from 1 g of pancreatic tissue were suspended in 10 ml oxygenated Hepes-buffered Ringer (HR-buffer) and equilibrated for 30 min at 37°C. After equilibration, acini were recovered by a brief centrifugation, washed once and resuspended in 40-45 ml leucine-free HR-buffer (when acini were incubated with ^{3}H-leucine) or containing all essential amino acids. Acini suspensions were immediately divided into 1 ml aliquots in Erlenmeyer flasks. A small aliquot from each preparation was mixed with an equal volume of 0.4% trypan blue in HR-buffer and exclusion of the dye by the cells, as an assessment of cellular viability, was tested. Preparations containing over 95% viable cells were used.

To measure the rate of protein synthesis in isolated acini, the reactions at 37°C were initiated with 4,5-^{3}H-leucine (0.5 μCi; 60 Ci/mmol; NEN, Boston, MA) in the absence (basal) and presence of increasing concentrations (10-1000 ng/ml) of highly purified exotoxin-A (generous gift from Dr. Barbara Iglewski, University of Oregon Health Sciences Center, Portland, OR). The reactions were arrested by the addition of 1 mM cycloheximide followed by an immediate centrifugation at 2000 x g. The supernatant (medium) and the pellet (cells) were processed separately as described below.

Proteins in the medium (hereafter referred to as "secretory" proteins) were precipitated with 10% trichloroacetic acid (TCA). The protein precipitates were washed five times with 5% TCA containing unlabeled leucine (1 mg/ml) and then dissolved in NaOH. The samples were counted for radioactivity as described elsewhere (8). The 2000 x g pellet (cells) was homogenized in 1 ml 10% TCA. The precipitates containing proteins (hereafter referred to as "cellular" proteins) were washed five times with 5% TCA-leucine solution, incubated in the same solution at 90°C for 20 min and finally dissolved in 1 N NaOH. An aliquot was counted for radioactivity and another aliquot was assayed for protein content (10).

In experiments in which enzyme secretion was measured, the isolated acini were suspended in HR-buffer containing all essential amino acids. Incubation at 37°C was initiated with exotoxin-A or an equivalent volume of HR-buffer, and terminated by centrifugation at 2000 x g. The supernatant (medium) was assayed for amylase (11), trypsinogen (12) and chymotrypsinogen (12) as described elsewhere (8). The 2000 x g pellet (cells) was dispersed in dispersing buffer (0.01 M Tris-HCl, pH 7.5 - 0.1% bovine serum albumin - 0.05 M $CaCl_2$ and 1% Triton-X-100) and centrifuged. The supernatant was assayed for amylase, trypsinogen and chymotrypsinogen.

RESULTS AND DISCUSSION

One of the least understood aspects of bacterial virulence in general is how a microorganism actually produces systemic illness and death of the infected host. Recently Pseudomonas aeruginosa exotoxin-A has emerged as a major candidate as a virulence factor, specifically in relation to systemic disease. Pseudomonas exotoxin-A is highly lethal for animals including subhuman primates and produces shock in dogs (13) and in rhesus monkeys (14). It has been shown serologically during human infection (15,16) and biochemically during experimental infection in mice (17) that pseudomonas exotoxin-A is released systemically. It is known that in addition to exotoxin Pseudomonas aeruginosa produces a large number of extracellular substances including pigments, hydrocyanic acid, proteolytic enzymes, phospholipase, slime and enterotoxin (18). Although the relative contribution of each of the factors in the pathogenesis of pseudomonas infection have not been fully elucidated, it is becoming increasingly clear that exotoxin-A plays an important role in the pathogenesis of the infection. Administration of purified exotoxin-A to intact mouse causes severe necrosis of the liver (19), accompanied by a dramatic fall in hepatic protein synthesis (6). A similar phenomenon occurs when mice are infected with exotoxin-A producing strain of pseudomonas but not when the animals are infected with the mutant strain, devoid of the toxin (20).

The data presented in this communication demonstrate that exotoxin-A exerts a direct toxic effect on the exocrine pancreatic cells, as evidenced by the diminished incorporation of ^{3}H-leucine into "cellular" (those remaining in the cells) and "secretory" (those released into the medium) proteins in isolated pancreatic acini by the toxin. Exposure of isolated acini to as little as 10 ng/ml of exotoxin-A for 3 h decreased the protein specific radioactivity (dpm/mg acinar protein) of "secretory" and "cellular" proteins by 25 and 9%, respectively, when compared with the corresponding basal controls (Fig. 1). No further reduction of protein specific radioactivities occurred with exotoxin-A doses between 10 and 100 ng/ml. Higher than 100 ng/ml of

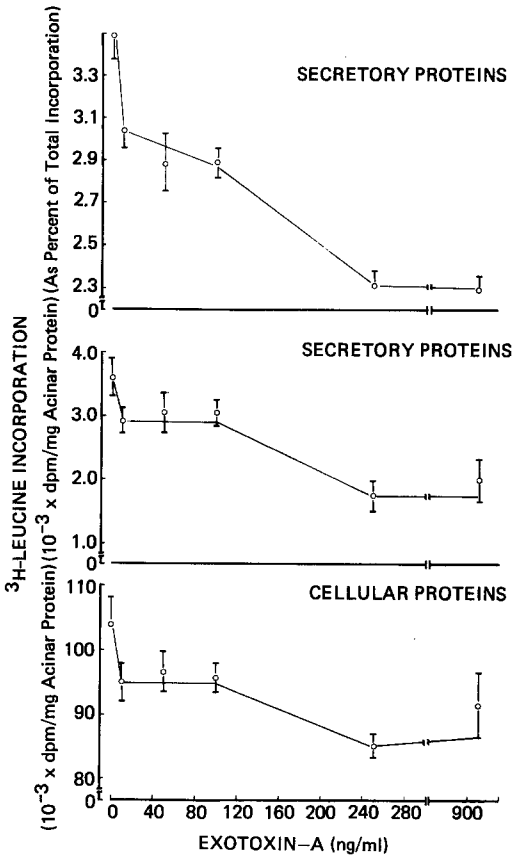

Fig. 1. Effect of increasing concentrations of exotoxin-A on the incorporation ^{3}H-leucine into "secretory" (those released into the medium) and "cellular" (those remaining in the cells) proteins in isolated dispersed pancreatic acini. Incubations were performed at 37°C for 3 h. The data on ^{3}H-leucine incorporation into "secretory" proteins were expressed as percent of total incorporation (upper panel) and as dpm/mg acinar protein (protein specific radioactivity; middle panel). Each value represents the mean ± SEM of duplicate determinations from 3 experiments.

exotoxin-A produced further reduction in ^{3}H-leucine incorporation into both "cellular" and "secretory" proteins (Fig. 1). However, the magnitude of reduction with each dose of exotoxin-A was found to be at least two times higher for "secretory" proteins than that of the "cellular" proteins, when compared with the corresponding basal controls. Thus, in the presence of 250 ng/ml exotoxin-A, the protein specific radioactivity of "cellular" and "secretory" proteins was decreased by 19 and 50%, respectively, as compared to the corresponding controls (Fig. 1). The comparatively larger reduction in the protein specific radioactivity of "secretory" proteins could not be considered artifactual. When ^{3}H-leucine incorporation into "secretory" proteins, was

expressed as percent of total incorporation, the results revealed a sharp decline in incorporation with increasing concentrations of exotoxin-A up to 250 ng/ml (Fig. 1). These observations suggest that in isolated pancreatic acini, exotoxin-A affects the synthesis of "secretory" proteins to a greater extent than the structural proteins.

The present finding of decreased acinar protein synthesis by exotoxin-A is similar to what has been observed by Fitzgerald et al (21) in mouse LM cell fibroblasts whose ability to incorporate ^{3}H-leucine into total proteins decreases considerably in the presence of 100 ng/ml of exotoxin-A.

In the next series of experiments, the effects of increasing concentrations (10 to 1000 ng/ml) of exotoxin-A on the secretion of digestive enzymes was investigated. The results are shown in Fig. 2. It was observed that exotoxin doses between 100 and 1000 ng/ml resulted in a steady decline in trypsinogen, chymotrypsinogen and amylase release from the isolated pancreatic acini. The observation of a near complete inhibition of chymotrypsinogen discharge, as opposed to 40 and 50% inhibition in trypsinogen and amylase release by the biggest dose of exotoxin-A, suggests that all digestive enzymes are not equally affected by the toxin. Such a postulation can be further substantiated by the observation that whereas low doses of exotoxin-A caused a slight increment in trypsinogen and amylase release, all doses of the toxin produced inhibition in chymotrypsinogen secretion. These observations not only show a selective effect of exotoxin-A on the release of digestive enzymes but also demonstrate nonparallel secretion of enzymes from isolated pancreatic acini. The parallelism between the profound inhibition of the synthesis of "secretory" proteins and enzyme release in the isolated acini by high doses of exotoxin-A suggests that the two processes may be coupled.

In summary, the current data demonstrate that exposure of isolated dispersed pancreatic acini to exotoxin-A results in inhibition of synthesis and secretion of proteins. It is concluded that the toxin exerts a direct toxic effect on the exocrine cells of the pancreas.

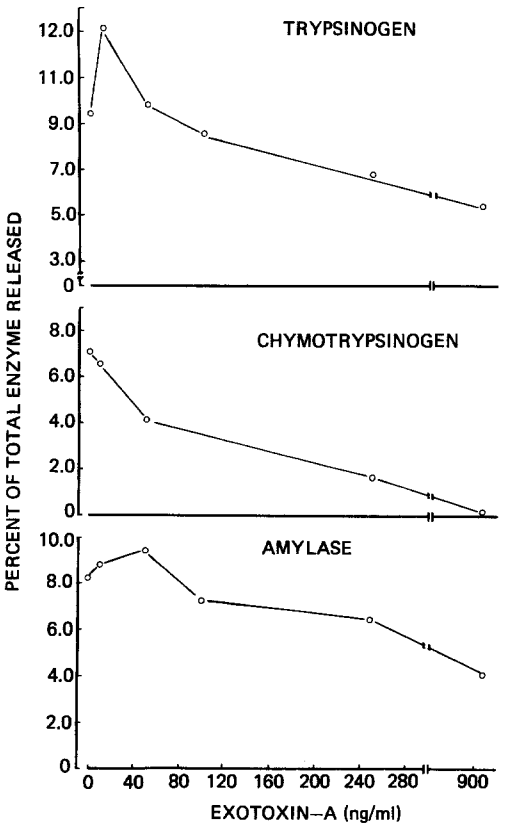

Fig. 2. Effect of increasing concentrations of exotoxin-A on the release of trypsinogen, chymotrypsinogen and amylase from isolated pancreatic acini. Incubations were performed at 37°C for 3 h. Results were expressed as percent of total enzyme released, taking into account, the enzyme released into the incubation and the residual activity present in the cells at the end of the incubation period. Zero-time values were subtracted from each experimental point. Each value represents the average of duplicate determinations from 2 experiments.

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